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Arbuscular mycorrhizae modify winter wheat root morphology and alleviate phosphorus deficit stress

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ABSTRACT

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Arbuscular mycorrhizal (AM) root colonization is known to have beneficial effects on plant growth especially under phosphorus (P) deficit conditions. The objectives of present study were: (i) to quantify changes in early wheat root development of AM-inoculated (AMI) and AM-free (AMF) roots under limited P availability; (ii) to assess possible mitigating effect of AM inoculation on photochemical efficiency under P deficit stress. AMI (inoculated with *Rhizophagus irregularis*) and AMF wheat plants were grown for 20 days in low (1 µmol/L) and high (50 µmol/L) P treatments. AM inoculation affected root morphology and shoot P concentration in low P treatment. AM inoculation alleviated reduction of the total root length in low P treatment, mainly due to an increase of fine roots length (< 0.5 mm). Contrastingly, shoot dry weight was reduced by AM inoculation in low P treatment. P deficiency decreased photochemical efficiency of wheat plants. However, due to increased sink capacity and facilitated nutrient concentrations AM inoculation alleviates phosphorus deficit stress and increased photochemical efficiency.

Keywords: plant macronutrient; root system; mutualism; chlorophyll fluorescence; shoot dry weight

Phosphorus (P) is known as the least available plant nutrient under most soil conditions and therefore represents a major limiting factor for crop production (Hinsinger 2001). Wheat root system characteristics are of fundamental importance to soil exploration and below-ground resource acquisition especially under suboptimal soil conditions (Manschadi et al. 2008). Root architectural traits such as shallower root growth angles, enhanced adventitious rooting, and greater dispersion of lateral roots, along with increased root hair number and length and mycorrhizal associations, represent adaptations that enhance soil exploration and lead to more efficient P acquisition (Lynch 2011). In addition, arbuscular mycorrhizal fungi (AM) has been widely studied as promising biofertilizer, especially under the P deficiency conditions (Pellegrino et al. 2015). Furthermore, root development is a highly plastic process which is influenced by plant endogenous characteristics (genetic control) and different external stimuli (environmental control) (Malamy 2005), and it is well known that P availability could act as an external signal with a profound impact on root system development (López-Bucio et al. 2003). Also, there is evidence that AM fungi could substantially change root architecture (Atkinson et al. 2003).

Therefore, the objectives of the present study were: (i) to quantify changes in early wheat root development of AM-inoculated and AM-free roots under limited P availability; (ii) to assess a possible mitigating effect of AM inoculation on photochemical efficiency under P deficit stress.

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MATERIAL AND METHODS

Plant material and cultivation. For this research, P-inefficient wheat cv. Sana was selected. Sana is a high yielding cultivar with increased harvest index, very responsive to intensive fertilization, and is widely used in Croatian winter wheat breeding programs (Kozumplik and Martinić-Jerčić 2000). Sterilized seeds (1 min in 70% (v/v) ethanol and 10 min in 5% sodium hypochlorite) were germinated on the moistened germination paper for three days at 4°C and two days at 20°C. Uniformly developed seedlings were transferred to 5 L plastic pots, filled with substrate made of 1:1 washed river sand and perlite. Plants were grown for 20 days in growing chamber under 20:15°C, 16:8 h, 70% relative air humidity, and 280 µmol/m^2/s photosynthetically active radiation (PAR). Pots were weighed two times per week and irrigated using the modified Magnavaca nutrient solution (Magnavaca et al. 1987) differing in P concentration, (pH 5.8) to maintain 70% of the medium field water capacity.

Experimental design. The experiment was set out as a randomized complete block design with two P concentration treatments, 1 µmol/L (low) and 50 µmol/L (high), and two AM treatments, AM-inoculated (AMI) and AM-free (AMF) plants. For the inoculation, 0.5 g (equivalent of 2000 spores) of Mycodrip (*Rhizophagus irregularis*, Symbiom LTD Lanškroun, Czech Republic) per pot was applied to the substrate mixture. AMF plants received the same amount of spore-free carrier. Each P × AM inoculation treatment combination was represented by 8 plants (pots).

Root system measurements. At the end of the experiment, roots were washed from the substrate and were scanned with an Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan). Images were analysed using WinRHIZO Pro (Regent Instruments Inc., Ville de Quebec, Canada) to determine total root length, root surface area, root volume, average root diameter, and length of the roots with different diameter. Roots with diameter 0–0.5 mm were defined as fine, and those with diameter 0.5–1.5 mm were defined as coarse. Root system depth was measured manually.

Root hair measurements. Three root segments (1 cm) per plant were cut off and stained using 0.05% Toluidine Blue dye. Root segments were immersed in distilled water to suspend the root hairs. Images were captured using a stereomicroscope (Leica EZ4W, Wetzlar, Germany), and root hair length and number were determined in WinRHIZO Pro.

AMF root colonization analysis. To determine the extent of AMF root colonization, 15 youngest root parts (1 cm) per plant were cleared with hot 10% KOH and acidified with 1 mol/L HCl. Root parts were stained with 0.05% Trypan Blue in lactoglycerol. Percentage of AMF root colonization (F%) was determined following Trouvelot et al. (1986) using a stereomicroscope (Leica EZ4W). A stained root segment with visible mycorrhizal structures is shown in Figure 1.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence measurements were performed 20 days after planting, using a Plant Stress Kit (Opti-Sciences, Inc., Hudson, USA) on the youngest fully developed leaf. To compensate for the leaf heterogeneity, three measurements per leaf blade were recorded and the average value was calculated. In dark-adapted leaves (overnight dark adaptation) the minimal fluorescence (F_0) was measured using 0.15 µmol/m^2/s PAR light flesh and maximal fluorescence (F_m) was measured using 1 s saturation pulse (4000 µmol/m^2/s PAR). In light-adapted leaves (after 3 h of actinic light) the steady state fluorescence (F_s), maximal fluorescence (F_m'), and minimal fluorescence (F_0') were determined. Based on those measurements, maximal quantum yield of PS 2 (F_v/F_m), actual quantum yield of PS 2 (YII), apparent electron transport rate (ETR), proportion of open PS 2

Figure 1. Mycorrhizal structures within the stained wheat (*Triticum aestivum*) roots inoculated with *Rhizophagus irregularis* (bar 150 µm)

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(qP), and non-photochemical quenching (NPQ) were calculated (Schreiber et al. 1994):

$$F_v/F_m = (F_m - F_0)/F_m;$$

$$Y(II) = (F_m' - F_s)/F_m';$$

$$ETR = Y(II) \times PAR \times 0.84 \times 0.5;$$

$$qP = (F_m' - F_s)/(F_m' - F_0');$$

$$NPQ = (F_m - F_m')/F_m'.$$

For the ETR estimation the average leaf light absorbance of 84%, and the portion of light provided to PS 2 of 50% was assumed.

Shoot dry weight and mineral concentration analysis. Shoots were dried at 70°C for 48 h and weighed for dry weight (DW) determination. Dried shoot tissue was ground, homogenized and digested in a microwave oven with nitric and perchloric acid (6:1). Shoot mineral concentration was determined using standard methods: phosphorus (P) using spectrophotometer, potassium (K) by flame photometer, calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) by atomic absorption spectrometry (Thermo Scientific, SOLAAR M Series AA Spectrometer, Waltham, USA). P uptake (μg) per plant was calculated by multiplying shoot DW by shoot P concentration.

Statistical analysis. Data were analysed using a SAS 9.3 statistical package (SAS Institute Inc., 2011). Mixed model ANOVA was performed and means pairwise differences were calculated by the Tukey-Kramer method. In the case of AM fungal colonization one-way ANOVA was used to test the differences between P treatments within AMI treatment, since no AM fungal structures were observed in the AMF treatment.

RESULTS AND DISCUSSION

AM fungal colonization and root traits. Although wheat is often considered as a non-responsive plant to AM inoculation, in the present study root colonization by AM fungi *R. irregularis* was relatively fast established (20 days of growth) and reached 32.6% in low P treatment (data not shown). High P treatment reduced percentage of AM fungi colonization to 13.8%, which was previously frequently shown (Li et al. 2006). No AM fungal structures were observed in the roots of plants grown in the AMF treatment.

Low P treatment caused reduction of the total root length, surface area and volume (Table 1), as well as reduction of fine and coarse roots length, and increase in root hair number (Table 2). These results are in line with the reduction in primary root growth, increase in lateral root development, and production of higher number of root hairs, described as fundamental root alternations under low P conditions (López-Bucio et al. 2003). However, AM inoculation alleviated reduction of the total root length in low P treatment (Table 1), mainly due to a significant (51.2%) increase of fine roots length (Table 2). These results indicate a positive effect of AM on root branching. A similar effect of AM on root branching was also described in maize (*Zea mays* L.) (Kaldorf and Ludwig-Müller 2000) and trifoliate orange (*Poncirus trifoliata* L. Raf.) (Yao et al. 2009). Root morphology was not affected by AMI in high P treatment, possibly due to low colonization frequency (only 13.8%). For example, Feldman et al. (2009) stated that plant

Table 1. Means (± standard deviation) and analysis of variance for the root system traits of AM-free (AMF) and AM-inoculated (AMI) wheat grown at 1 μmol/L (low) and 50 μmol/L (high) phosphorus (P) treatments

Treatment	Depth (cm)		Total length (cm)		Surface area (cm^2)		Volume (mm^3)		Average diameter (mm)	
	high	low	high	low	high	low	high	low	high	low
AMI	34.1 ± 2.2^a	32.8 ± 2.5^a	350.7 ± 51.9^a	264.2 ± 23.5^a	42.8 ± 6.5^a	30.6 ± 3.8^a	0.45 ± 0.07^a	0.31 ± 0.06^a	0.42 ± 0.05^a	0.40 ± 0.03^a
AMF	34.7 ± 2.4^a	34.8 ± 1.7^a	334.3 ± 42.6^a	195.8 ± 25.1^b	44.4 ± 7.7^a	27.4 ± 3.6^a	0.48 ± 0.11^b	0.30 ± 0.06^a	0.43 ± 0.04^a	0.43 ± 0.03^a
$\bar{x}$	34.4	33.8	342.5	230.0	43.6	29.0	0.46	0.31	0.43	0.42
P	ns		*		***		***		ns	
AM	ns		***		ns		ns		ns	
P × AM	ns		*		ns		ns		ns	

*P < 0.05; **P < 0.01; ***P < 0.001; ns – non significant. Different letters indicate AM (arbuscular mycorrhizal) treatment means differences within P treatments (P = 0.05)

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Table 2. Means (± standard deviation) and analysis of variance for length of the fine and coarse roots and root hair number and the length of AM-free (AMF) and AM-inoculated (AMI) wheat grown at 1 µmol/L (low) and 50 µmol/L (high) phosphorus (P) treatments

Treatment	Length (cm) of fine roots (0.0–0.5 mm)		Length (cm) of the coarse roots (0.5–1.2 mm)		Root hair number (per 1 cm of root length)		Root hair length (mm)	
	high	low	high	low	high	low	high	low
AMI	260.1 ± 39.4^a	195.0 ± 16.9^a	90.7 ± 19.0^a	69.2 ± 7.3^a	59.2 ± 5.7^a	59.4 ± 4.3^b	0.76 ± 0.14^a	0.79 ± 0.17^a
AMF	239.2 ± 35.8^a	129.0 ± 20.1^b	95.0 ± 12.4^a	66.8 ± 7.3^a	56.9 ± 5.6^a	69.6 ± 3.2^a	0.80 ± 0.17^a	0.81 ± 019^a
$\bar{x}$	249.7	162.0	92.9	68.0	57.8	63.5	0.78	0.80
P	***		***		*		ns	
AM	**		ns		ns		ns	
P × AM	*		ns		*		ns	

*P < 0.05; **P < 0.01; ***P < 0.001; ns – non significant. Different letters indicate AM (arbuscular mycorrhizal) treatment means differences within P treatments (P = 0.05)

benefits from the AM were not evident under the threshold of about 30% root colonization. In addition, a significant P × AM interaction for root hair number was found. AMF plants produced higher number of root hairs (for 17.2%) compared to AMI plants in low P treatment (Table 2). Root hair development is regulated by P availability in a dose-dependent manner (Bates and Lynch 2001), thus higher number of root hairs found in AMF plants grown in low P treatment could be explained by lower ability of AMF plants for the P uptake.

Shoot dry weight and mineral concentrations. AMI increased shoot P concentration for 13.8% in low P treatment, whereas these differences were not significant in high P treatment (Table 3). However, there was no difference in P uptake between AMI and AMF plants (Table 3). These results could be explained by a significant reduction in shoot DW of AMI plants compared to AMF plants grown in low P treatment. Namely, in low P treatment shoot DW of AMI plants decreased for 12.6% compared to AMF plants (Table 3). Li et al. (2006) showed that AM can substantially contribute to the P acquisition (more than 50%) during the early stages of wheat growth. In addition, mycorrhizal-induced growth depression in plants has been frequently observed (Jin et al. 2017). AM fungi receive photosynthetic products from plant (Treseder 2013) and thus represent sink in the plants phloem transport, however in

Table 3. Means (± standard deviation) and analysis of variance for shoot dry weight (DW), plant phosphorus (P) uptake and shoot macroelements concentration of AM-free (AMF) and AM-inoculated (AMI) wheat grown at 1 µmol/L (low) and 50 µmol/L (high) phosphorus treatments

Treatment	P (g/kg)		K (g/kg)		Ca (g/kg)		Mg (g/kg)		DW (mg/plant)		P uptake (µg/plant)	
	high	low	high	low	high	low	high	low	high	low	high	low
AMI	2.66 ± 0.07^a	1.44 ± 0.05^a	69.2 ± 1.2^a	63.2 ± 3.0^a	6.3 ± 0.4^a	6.4 ± 0.2^a	2.2 ± 0.3^a	2.3 ± 0.2^a	55.5 ± 3.1^a	43.0 ± 4.5^b	148.3 ± 4.0^a	59.8 ± 4.7^a
AMF	2.70 ± 0.10^a	1.26 ± 0.04^b	71.3 ± 2.0^a	61.5 ± 3.1^a	5.2 ± 0.3^b	5.3 ± 0.5^b	2.2 ± 0.5^a	2.1 ± 0.3^a	55.8 ± 4.1^a	49.2 ± 1.5^a	149.9 ± 5.5^a	61.7 ± 1.2^a
$\bar{x}$	2.68	1.35	70.3	62.4	6.2	6.4	2.2	2.2	55.7	46.1	149.1	60.8
P	***		**		ns		ns		***		***	
AM	ns		ns		*		ns		*		ns	
P × AM	*		ns		ns		ns		*		ns	

*P < 0.05; **P < 0.01; ***P < 0.001; ns – non significant. Different letters indicate AM (arbuscular mycorrhizal) treatment means differences within the P treatments (P = 0.05)

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case of the present study, it seems that a decrease in shoot DW of AMI plants grown in low P treatment is also related to increased carbon investment in root growth. High P treatment caused a 12.6% increase in shoot K concentration (Table 3), which could be explained by bigger root system of plants grown in high P treatment (Table 1). AM colonization increased average shoot Ca concentration and did not affect Mg (Table 3) as well as micronutrient concentration (data not shown). Sedláček et al. (2013) also describes a significant increase of Ca concentration in grapevine leaves after inoculation with AM fungi.

Chlorophyll fluorescence. Low P treatment decreased Y(II), ETR, and qP for both AMI and AMF plants (on average for 32, 28.4, and 28.8%, respectively); however this reduction was less pronounced in AMI plants (Table 4). Similar reduction of the ETR and Y(II) in P deficit plants was found by Hernández and Munné-Bosch (2015). These results indicate that AMI in low P treatment increases proportion of open PS 2, and thus increases the proportion of energy used in photochemical reaction, and increases the ETR. A possible explanation of this effect is increased P concentration and/or increased sink capacity of AMI plants compared to AMF plants grown in low P treatment. Under P-limited conditions the production of assimilates is limited (Rychter and Rao 2005), and this could cause feedback limitation of light reactions. Hence, Quick and Mills (1988) stated that inorganic phosphate (Pi) incorporated in the sugar phosphate end-products of photosynthesis needs to be recycled in order to sustain photophosphorylation. Thus, increased sink capacity of AMI plants could increase sugar utilization and increase the rate of Pi recycling.

As was stated by Gregory (2006) and references cited there, AM inoculation contributes to an increase in P utilization by increasing solubility of sparingly soluble inorganic and organic P compounds, and thus reduces the need for P fertilizers application, which has positive economic and environmental effects. However, removal of plant biomass and associated phosphorus, without returning it in forms of P fertilizers, has great long-term impacts on depletion of inorganic P from mineral surfaces (Simpson et al. 2012) and changes in structure of soil bacterial community (Adair et al. 2013).

Results of this study show that AM colonization affects early wheat root morphology by promoting root branching and development of fine roots, and increases shoot P concentration under P deficient conditions. Alternations in root morphology caused by AM colonization were explained by modified nutritional status of the host plant and/or altered levels of phytohormones (Yao et al. 2009). In the present study, AMI improved shoot P concentration, which could support nutritional mechanisms theory of the root morphology alternations. However, a decrease in shoot DW along with beneficial effects on photochemical efficiency of AMI plants under P deficit conditions point to the systemic and integrated plant response at the whole-plant level.

Table 4. Means (± standard deviation) and analysis of variance for chlorophyll fluorescence of AM-free (AMF) and AM-inoculated (AMI) wheat grown at 1 μmol/L (low) and 50 μmol/L (high) phosphorus (P) treatments

Treatment	F_v/F_m		Y(II)		ETR		qP		NPQ	
	high	low	high	low	high	low	high	low	high	low
AMI	0.82 ± 0.02^a	0.82 ± 0.02^a	0.50 ± 0.03^a	0.39 ± 0.02^a	43.5 ± 6.0^a	39.1 ± 4.9^a	0.59 ± 0.04^a	0.47 ± 0.03^a	1.88 ± 0.14^a	1.99 ± 0.23^a
AMF	0.83 ± 0.01^a	0.81 ± 0.02^a	0.50 ± 0.05^a	0.30 ± 0.02^b	48.1 ± 7.8^a	26.4 ± 4.5^b	0.60 ± 0.06^a	0.36 ± 0.02^b	1.90 ± 0.27^a	2.10 ± 0.26^a
$\overline{x}$	0.825	0.815	0.50	0.34	45.8	32.8	0.59	0.42	1.89	2.05
P	ns		***		***		***		ns	
AM	ns		**		ns		**		ns	
P × AM	ns		**		**		**		ns	

*P < 0.05; **P < 0.01; ***P < 0.001; ns – non significant. Different letters indicate AM (arbuscular mycorrhizal) treatment means differences within the P treatments (P = 0.05). F_v/F_m – maximal quantum yield of PS 2; YII – actual quantum yield of PS 2; ETR – electron transport rate; qP – proportion of open PS 2; NPQ – non-photochemical quenching

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The age of plants might have a significant role in the results of this experiment and the prolonged effect of AM and P availability on the studied traits could be more severe, especially on the higher P uptake of AMI plants under low P treatment.

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